



Malaria Research Lead Programme

**Laboratory Bioassay To Determine The Efficacy Of An
Essential Oil in Formulation, As A Mosquito Repellent
Lee-Chem Laboratories
2008**

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Medical Research Council

The work described in this report is being carried out in the Durban laboratories of the Malaria Research Programme of the Medical Research Council and was commissioned for Lee-Chem Laboratories.

Date: 13 March 2008

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Objective

This study was conducted to determine if an essential oil has a repellent effect against the malaria mosquito.

Materials and Methods

Repellency tests had been conducted in accordance with the standard WHO protocol and using the target species, *Anopheles arabiensis* (WHO, 1996).

The rodent *Mastomys coucha* was the test animal used for the screening of the samples for repellency activity. Ethical approval for the use of *Mastomys* in these trials was sought from the MRC's Ethics Committee for Research on Animals.

Each adult *Mastomys* had been weighed individually, and injected intraperitoneally with sodium pentobarbital at a rate of 1ml per 2.25 kg. The anaesthetized rodents were shaven on the ventral surface and the product was applied.

Paper cups (500ml) were modified by replacing the base of the cup with mosquito netting held in place with a rubber band and covering the mouth of the cup with transparent plastic film.

The trial comprised of five tests namely, one sample, a repeat test, a positive control (DEET) and two negative controls.

Thirty unfed 4-day old *Anopheles arabiensis* females was introduced into a cup and held in contact with exposed skin of each rodent. Mosquito activity was observed through the transparent plastic film. After a period of two minutes the number of mosquitoes probing had been recorded. The mosquitoes exposed to the product had been monitored one hour post exposure to determine if the product had induced a knockdown effect.

The rodent was returned to the animal facility and allowed to recover from the effects of the anesthetic. Each rodent was monitored for 7 days for adverse reactions to the essential oil formulation.

Results

Table one

Test sample	2minute Repellent effect %
03.03.08.001 A with 10% BP1	98
03.03.08.001 B is the one with no BP 1 for control-NEGATIVE	100
03.03.08.001 B is the one with no BP 1 for control-NEGATIVE	100
DEET	100

Discussion

A cream base using an essential oil as an active ingredient had been tested for repellent activity against the major vector of malaria, the *Anopheles arabiensis* mosquito.

The negative controls tested had been the base cream which did not contain the essential oil. The positive control was DEET (N, N-diethyl-m-toluamide) a known repellent proven to provide an individual with complete protection against mosquitoes.

All trials were conducted in duplicate to ensure consistency amongst results.

The criteria set for determining high repellent effect was 80-100% repellency in accordance with the positive control.

Referring to Table one, sample **A** had produced an encouraging 98 percent repellent effect.

The positive control results have shown an expected one hundred percent repellency whilst the negative controls conducted had exhibited a surprisingly high repellent effect equal to that of the positive control. No knockdown effect (1 hour post exposure) had been observed with any of the samples tested.

Mosquitoes have very sensitive olfactory chemoreceptors on their antennae which become stimulated by very distinct odors and depending on the type of odor it may either increase or decrease attractiveness to the host.

This behavior of reducing the attraction of mosquitoes to their host had been observed during the negative control trial.

As an additional control step prior to the application of every sample, each cup of mosquitoes were allowed to become acclimatized to the test animal for a short period. A large number of mosquitoes had initially been attracted to the test animal, however, once the negative control had been applied, the activity within the cup had increased but the number of landings on the treated area decreased considerably. The mosquitoes had been affected by the cream base and were unable to locate the test animal.

Although the test sample A had produced a significant repellent effect against the *Anopheles arabiensis* mosquito, it cannot be concluded that the repellent effect was due solely to the activity of the essential oil. Results have indicated that the cream base contained in all samples may be responsible for, or contributes to the high repellent effect of the product.

References

WHO,CTD/WHOPES/IC/96.1 Protocols for laboratory and field evaluation of Insecticides and Repellents